

## **Methanotrophic activity in hyperalkaline waters of the Kizildag ophiolite complex (Turkey)**

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The low temperature serpentinisation of ophiolites is a source of methane, which is an important greenhouse gas. The large Kizildag ophiolitic body outcrops in the Hatay region (southern Turkey). It belongs to the peri-Arabian ophiolite belt including the Troodos (Cyprus), Baër-Bassit (Syria) and Semail (Oman) ophiolites in the eastern Mediterranean region, which are the remnants of the Southern Tethys oceanic lithosphere. Hitherto six groups of hyperalkaline springs and one cold gas seep were sampled in the area and analysed for the geochemistry of the free or dissolved gases. The chemical composition of the gases shows a great variability with the dominant gases being always either  $H_2$ ,  $CH_4$  or  $N_2$  with concentrations in the range  $<0.001$ -60.5%, 0.003-94.2% and 1.3-99.5%, respectively. Notwithstanding this great variability, chemical and isotopic parameters confirmed a prevailing derivation from low temperature serpentinization processes and point to a dominant abiotic origin for  $H_2$ ,  $CH_4$  and light hydrocarbons. A few samples could be affected by a biogenic contribution for methane, while others show clear effects of methane oxidation. Although it has recently been hypothesized that life on Earth could have been originated in hyperalkaline waters related to serpentinization of ophiolitic rocks, such environments have to be considered as extreme for microbial life, because of the high pH and the very low levels of certain nutrients. On the other hand, the presence of gases like  $H_2$  and  $CH_4$ , originating from the serpentinization processes, represents a highly effective energy source for specialized microbial species. To acquire knowledge of biological processes possibly affecting these gases, molecular analyses were carried out in five springs belonging to three of the previously identified groups of hyperalkaline springs, in order to assess the diversity of bacterial communities and the presence of methanotrophs. Total DNA was extracted from the samples and used as template for Ribosomal Intergenic Spacer Analysis (RISA), targeting the bacterial 16S-23S ribosomal spacer; samples were also probed by PCR for the *pmoA* gene, encoding the key enzyme of methane oxidation. Despite the extreme conditions and scarce level of nutrients, each sample was characterised by a specific and simple banding pattern, with a negligible number of shared bands. Sequencing of excised DNA bands is in progress for bacterial identification. Three samples were also positive for the presence of *pmoA*, confirming the presence of methanotrophs. Such finding was consistent with gas analyses that showed clues of microbial methane oxidation in the isotopic ratio. The presence of methanotrophs in these hyperalkaline springs highlights the extraordinary capability of the methanotrophs to adapt to extreme conditions and suggests a role in limiting the global impact of methane emissions in such natural sources. Understanding  $CH_4$  and  $H_2$  formation and removal processes at low temperature serpentinisation, along with the study of microbial community thriving in hyperalkaline waters, might unveil insights on events occurring in primordial conditions and features of primordial bacteria, since such environments can be considered as the mirror of the early Earth at the time of the origin of life.

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